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Bad Bocklet, 04 June 2007

IN-VITRO ASSESSMENT
OF THE
ACUTE IRRITATION POTENTIAL
OF
“ROVISOME Biotin”
TO MUCOUS MEMBRANES
WITH THE
HEN'S EGG CHORIOALLANTOIC MEMBRANE TEST
(= HET-CAM)

- Report to ROVI Cosmetics - L+S-No.: 05652967 - page 1 of 8 pages -

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1. General Information

Client: ROVI Cosmetics International GmbH
Breitwiesenstr. 1
36381 Schlüchtern

Test item: "ROVISOME Biotin"

L+S-No.: 05652967

Reference from: May 22, 2007

Arrival of the test item: May 23, 2007

Start of study: May 25, 2007

End of study: June 04, 2007

Test performance: Ms Alexandra Hofmann, MTA

Study director: Dr Andreas Schlösser, Dipl Biol

Quality-assurance unit: Ms Melanie Braun, Biological Technician

Sponsor's monitor: Mr Michael Sacher

Date: June 04, 2007



2. Object

Object of this assay is to assess the acute irritation potential of a test item to mucous membranes in analogy to the Draize Eye Test. For this purpose a definite dose of the test item is applied to the chorioallantoic membrane (CAM) of fertilized and incubated hen's eggs.

3. Material and Methods

3.1 Material

Eggs:	hen's eggs (breed: White Leghorn, Art. Nr. 40050, Fa. Lohmann, Tierzucht, D-27454 Cuxhaven)
Incubation:	9 days, temperature $37,5 \pm 0,5$ °C; rel. humidity 55 ± 7 %
Reference item:	Texapon ASV 5%, Art. Nr. 547700, Fa. Henkel KGaA, D-40191 Düsseldorf (category of irritation: moderately irritating, tested according to OECD 405 in vivo, this item has not to be declared irritating according to the German "Gefahrstoffverordnung")
Test item:	"ROVISOME Biotin", L+S-Nr.: 05652967

3.2 Methods

The test was performed along the lines of:

STEILING W; BRACHER M; COURTELLEMONT P; DE SILVA O (1999):

The HET-CAM, A useful in vitro assay for assessing the eye irritation properties of cosmetic formulations and ingredients.

Toxicology In Vitro 13, 375 - 384

All eggs are incubated for nine days (blunt end upwards); their blunt ends are then (day 10) illuminated with a candling lamp. During candling the air space outlined at the blunt end of the eggs is marked. The egg shell is scratched along the marked line with the end of gooseneck tweezers, then the shell is prepared off. The white egg membrane is moistened with a few milliliters of physiological saline.

Preliminary tests of the test item and the reference item (Texapon ASV 5%) are carried out with one prepared egg each in an end-point assessment. The white egg membrane is carefully removed and the reference item as well as the test item are applied to the CAM in a dose of 300 µl. After application a chronometer is started and after 30 seconds, the test item is carefully rinsed off the CAM with physiological saline. Because any blood present may also be rinsed off the CAM, a weak hemorrhage could be overlooked immediately after rinsing. In order to avoid this, the CAM should first be assessed some 30 seconds after rinsing. The effects are evaluated semi-qualitatively from 0 (no reaction) to 3 (strong reaction).



Each item that is to be tested in the **main test** is applied to six eggs in an endpoint assessment. For the procedure see above *Preliminary tests*. All irritation parameters are calculated but for classification only the most impressive irritation parameter for each of six eggs is summed up (see below *Scoring system*).

3.3 Scoring system

The results have to be interpreted as follows:

The irritation value of the reference item Texapon ASV 5% has to be assessed as „moderately irritating“.

An item can be classified as irritating if at least one reaction type is assessed at least as moderate (sum of all scores [S] > 12 < 16) after 30 seconds. Severe in vivo effects are expected if one type is classified as strong (sum of all scores ≥ 16). In all other cases the expected irritation property of the test item in vivo is low.

Scoring System

Score [S]	Categories of irritation in vivo
< 6	„slightly irritating“
≥ 6 bis ≤ 12	„moderately irritating“
> 12 bis < 16	„irritating“
≥ 16	„severely irritating“



4. Results

For a more detailed interpretation of the results see also the appendix.

Item	hemorrhage (score of six eggs)	lysis (score of six eggs)	coagulation (score of six eggs)	Irritation value [S] = score of the most impressive irritation parameter
Texapon ASV, 5%	6	0	0	6
ROVISOME Biotin	0	7	0	7

5. Conclusions

For the test item "**ROVISOME Biotin**" an irritation value of 7 was determined. On this account the test item can be classified in vivo as „moderately irritating“.

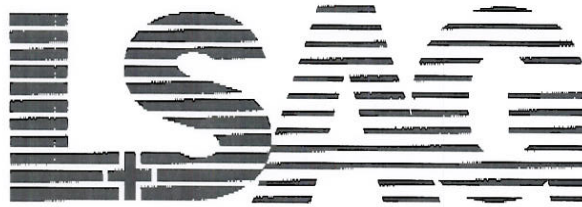
The reference item Texapon ASV 5% was assessed as „moderately irritating“ (irritation value of 6) what demonstrated the validity of the test procedure.

A handwritten signature in black ink, appearing to read 'Schlösser'.

Dr Andreas Schlösser
- head of department -

A handwritten signature in black ink, appearing to read 'Matin'.

Dipl - Biol Anastasija Matin
- head of department -



Appendix 1

Protocol HET-CAM

L+S-No.: 05652967
Test item: ROVISOME Biotin
Arrival: 23.05.2007
Start of study: 25.05.2007

Sponsor: ROVI Cosmetics International GmbH
Specification: none
Reference from: 22.05.2007
End of study: 04.06.2007

The test was conducted along the line of:

STEILING W; BRACHER M; COURTELLEMONT P; DE SILVA O (1999):

The HET-CAM, A useful in vitro assay for assessing the eye irritation properties of cosmetic formulations and ingredients.

Toxicology In Vitro 13, 375 - 384

Dilution / Concentration of test item: 100%

Time of application: 30 sec

pH - value of test item: 5,5

Test item	Type of reaction			Irritation value [S] Score of most impressive irritation parameter	Categorie of irritation
	Hemorrhage (score of six eggs)	Lysis (score of six eggs)	Coagulation (score of six eggs)		
"ROVISOME Biotin"	0	1	0		
	0	1	0		
	0	1	0		
	0	2	0		
	0	1	0		
	0	1	0		
Sum	0	7	0	7	„moderately irritating“
Texapon ASV 5%	1	0	0		
	1	0	0		
	1	0	0		
	1	0	0		
	1	0	0		
	1	0	0		
Sum	6	0	0	6	„moderately irritating“



Statement of QAU

This report provides a correct and faithful record of the results obtained.

A handwritten signature in black ink, appearing to read 'Melanie Braun'.

Melanie Braun
QAU

04.06.07

Date